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JC10 Rec'd PCT/PTO 28 JAN 2002

| FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE<br>TRANSMITTAL LETTER TO THE UNITED STATES<br>DESIGNATED/ELECTED OFFICE (DO/EO/US)<br>CONCERNING A FILING UNDER 35 U.S.C. 371 | ATTORNEY'S DOCKET NUMBER:<br>2503-1003<br>U.S. APPLN. NO. (If known, see 37 CFR 1.5)<br>10/048120 |
|---|---|

| INTERNATIONAL APPLICATION NO.:<br>PCT/EP00/07102 | INTERNATIONAL FILING DATE:<br>25 JULY 2000 | PRIORITY DATE CLAIMED:<br>26 JULY 1999 |
|---|---|---|

TITLE OF INVENTION: USE OF ORTHOESTERS FOR THE SYNTHESIS OF CHIRAL ACIDS IN BIOCATALYZED IRREVERSIBLE ESTERIFICATION PROCESSES

APPLICANT(S) FOR DO/EO/US: Raffaele MORRONE, Giovanni NICOLOSI, Mario PIATTELLI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. [ ] has been transmitted by the International Bureau. (see attached copy of PCT/IB/308)
   - c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   - a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. [ ] have been transmitted by the International Bureau.
   - c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   - d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Item 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

International Search Report
PCT/IPEA/409
Application Data Sheet

Abstract of the Disclosure on a Separate Sheet

| U.S. APPLICATION NO (if known, see 37 CFR 1.5) 10/048120 | INTERNATIONAL APPLICATION NO. PCT/EP00/07102 | ATTORNEY'S DOCKET NO 2503-1003 |
|---|---|---|

| | CALCULATIONS PTO USE ONLY |
|---|---|
| 17. [X] The following fees are submitted: | |
| **BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):** Neither international preliminary examination fee (37 CFR1.482) nor international search fee (37 CFR1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... $ 1,040.00 | |
| International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $ 890.00 | |
| International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $ 740.00 | |
| International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 710.00 | |
| International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00 | |
| **ENTER APPROPRIATE BASIC FEE AMOUNT =** | $ 890.00 |
| Surcharge of $130.00 for furnishing the oath or declaration later than 30 months from the earliest claimed priority date (37 CFR 1.492(e)). | $ 130.00 |

| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | $ |
|---|---|---|---|---|
| Total claims | 9 - 20 = | 0 | X $18.00 | $ |
| Independent claims | 2 - 3 = | 0 | X $84.00 | $ |
| MULTIPLE DEPENDENT CLAIMS(S) (if applicable) | | | + $280.00 | $ |

| | | |
|---|---|---|
| **TOTAL OF ABOVE CALCULATIONS =** | $ | 1,020.00 |
| Reduction of ½, if applicant is entitled to Small Entity status under 37 CFR 1.27. + | $ | 510.00 |
| **SUBTOTAL =** | $ | 510.00 |
| Processing fee of $130 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR1.492(f)). | $ | |
| **TOTAL NATIONAL FEE =** | $ | 510.00 |
| Fee for recording the enclosed assignment (37 CFR1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property + | $ | |
| **TOTAL FEES ENCLOSED =** | $ | 510.00 |
| | Amount to be refunded: | |
| | charged: | |

a. [X] A check in the amount of $ **510.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. **25-0120** in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1 17, or credit any overpayment to Deposit Account No. **25-0120**. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO

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January 28, 2002

By Benoît Castel
Benoît Castel
Attorney for Applicants
Registration No. 35,041

**Application Data Sheet**

**Application Information**

| | |
|---|---|
| Application Type:: | Regular |
| Subject Matter:: | Utility |
| Suggested Classification:: | |
| Suggested Group Art Unit:: | |
| CD-ROM or CD-R?:: | None |
| Number of CD disks:: | |
| Number of Copies of CDs:: | |
| Sequence Submission?:: | None |
| Computer Readable Form (CRF):: | No |
| Number of copies of CRF:: | 0 |
| Title:: | USE OF ORTHOESTERS FOR THE SYNTHESIS OF CHIRAL ACIDS IN BIOCATALYZED IRREVERSIBLE ESTERIFICATION PROCESSES |
| Attorney Docket Number:: | 2503-1003 |
| Request for Early Publication?:: | No |
| Request for Non-Publication?:: | No |
| Suggested Drawing Figure:: | |
| Total Drawing Sheets:: | 2 |
| Small Entity?:: | Yes |
| Latin Name:: | |
| Variety Denomination Name:: | |
| Petition Included?:: | No |
| Petition Type:: | |
| Licensed US Gov't Agency:: | |
| Contract or Grant Numbers:: | |
| Secrecy Order in Parent Appl.?:: | No |

**Applicant Information**

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State or Province of Mailing Address::
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City of Mailing Address:: VALVERDE
State or Province of Mailing Address::
Country of Mailing Address:: ITALY
Postal or Zip Code of Mailing Address:: I-95028

**Correspondence Information**

Correspondence Customer Number:: 000466

**Representative Information**

| | |
|---|---|
| Representative Customer Number:: | 000466 |

**Domestic Priority Information**

| Application:: | Continuity Type:: | Parent Application:: | Parent Filing Date:: |
|---|---|---|---|
| This application | National Stage of | PCT/EP00/07102 | 7/25/00 |
| | | | |

**Foreign Priority Information**

| Country:: | Application Number:: | Filing Date:: | Priority Claimed:: |
|---|---|---|---|
| ITALY | ME99A000005 | 7/26/99 | Yes |
| | | | |

**Assignment Information**

Assignee Name::

Street of Mailing Address::

City of Mailing Address::

State or Province of Mailing Address::

Country of Mailing Address::

Postal or Zip Code of Mailing Address::



Initial 1/28/02

PATENT
2503-1003

**IN THE U.S. PATENT AND TRADEMARK OFFICE**

In re application of: Raffaele MORRONE et al.

Appl. No.: Group:

Filed: January 28, 2002 Examiner:

For: USE OF ORTHOESTERS FOR THE SYNTHESIS OF CHIRAL ACIDS IN BOICATALYZED IRREVERSIBLE ESTERIFICATION PROCESSES

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents
Washington, DC 20231

January 28, 2002

Sir:

The following preliminary amendments and remarks are respectfully submitted in connection with the above-identified application.

IN THE CLAIMS:

Please amend the claims as follows:

--4. (amended) A process as claimed in claim 1, wherein said esterification reaction is carried out at a temperature of 0-50°C, preferably at 45°C.--

--5. (amended) A process as claimed in claim 1 comprising the step of adding the reaction mixture with an amount of water or of a alcohol with 1-8 carbon atoms

equivalent to 1-5% mols compared with the mols of said chiral carboxylic acid.--

--6. (amended) A process as claimed in claim 1, wherein in said esterification reaction the meso form of a bicarboxylic acid is used as substrate.--

--7. (amended) A process as claimed in claim 1, wherein said carboxylic acid is selected from (±)-(R,S)-2-(2-fluoro-4-biphenyl)-propionic, (±)-(R,S)-2-(3-benzoylphenyl)-propionic, (±)-(R,S)-2-(4-isobutylphenyl)-propionic, (±)-(R,S)-2-[4-(1-oxo-2-isoindolinyl)phenyl] propionic, (±)-(R,S)-2-[4-(2-thenoyl)phenyl]-propionic, (±)-(R,S)-2-(6-methoxy-2-naphthyl)-propionic acids. --

REMARKS

Claims 1-9 are pending in the present application.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly requested.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

YOUNG & THOMPSON

Benoit Castel

Benoit Castel, Reg. No. 35,041

745 South 23rd Street
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Telephone (703) 521-2297

BC/ia
Attachments

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

IN THE CLAIMS:

The claims have been amended as follows:

4. (amended) A process as claimed in ~~any one of the above claims,~~ claim 1, wherein said esterification reaction is carried out at a temperature of 0-50°C, preferably at 45°C.

5. (amended) A process as claimed in ~~any one of the above claims~~ claim 1 comprising the step of adding the reaction mixture with an amount of water or of a alcohol with 1-8 carbon atoms equivalent to 1-5% mols compared with the mols of said chiral carboxylic acid.

6. (amended) A process as claimed in ~~any one of the above claims,~~ claim 1, wherein in said esterification reaction the meso form of a bicarboxylic acid is used as substrate.

7. (amended) A process as claimed in ~~the above claims 1-6,~~ claim 1, wherein said carboxylic acid is selected from (±)-(R,S)-2-(2-fluoro-4-biphenyl)-propionic, (±)-(R,S)-2-(3-benzoylphenyl)-propionic, (±)-(R,S)-2-(4-isobutylphenyl)-~~isobutylphenyl)~~-propionic, (±)-(R,S)-2-[4-(1-oxo-2-isoindolinyl)phenyl] propionic, (±)-(R,S)-2-[4-(2-thenoyl)phenyl]-propionic, (±)-(R,S)-2-(6-methoxy-2-naphthyl)-propionic acids.

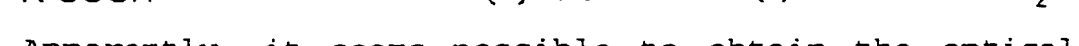
Abstract of the Disclosure

A process for the resolution of enantiomeric mixtures of a chiral carboxylic acid, including an esterification reaction of the carboxylic acid in an organic solvent, in the presence of a stereoselective hydrolase, characterized in that an orthoester of the formula: $R^1$-$C(OR^2)_3$, in which $R^1$ is selected from H and $C_1$-$C_4$alkyl and $R^2$ is $C_1$-$C_8$alkyl or -$CH_2$-$C_6$-$_{10}$aryl, is used as the esterification reactive.

# THE USE OF ORTHOESTERS FOR THE SYNTHESIS OF CHIRAL ACIDS IN BIOCATALYZED IRREVERSIBLE ESTERIFICATION PROCESSES

Enantiomerically pure chiral compounds are increasingly required in recent times, as these compounds may be used in a number of different fields (biomedical, agroalimentary, special materials and the like). Racemic chiral acids may be resolved by means of esterification in organic solvent, catalyzed by a hydrolase (lipase, esterase, protease), as illustrated for example in IT 1 274 482 and IT 1 275458.

When a racemic acid RCOOH is reacted with an alcohol R'OH in the presence of a hydrolase with R-stereopreference, this enantiomer will be the fast reacting one, undergoing more rapidly the esterification, so that the unreacted acid will enrich in the S enantiomer, according to the following scheme:




Apparently, it seems possible to obtain the optically pure S isomer simply by extending the conversion to a sufficiently high value. However the reversibility of this reaction makes the situation complicated, as the R enantiomer, which is the faster formed one, is also the one more easily undergoing hydrolysis, to the detriment of the optical purities of both the R ester and the S acid residue (Chen, C. S.; Wu, S. H.; Girdaukas, G. and SiH, C. *J. Am. Chem. Soc.* 1987, *109*, 2812 – 2817).

The above mentioned limits are also found in the desymmetrization of polycarboxylic acids meso-forms, when carrying out their enantiotoposelective esterification in the presence of hydrolase.

Many approaches have been proposed to overcome the problems connected with the reversibility of the esterification reaction:

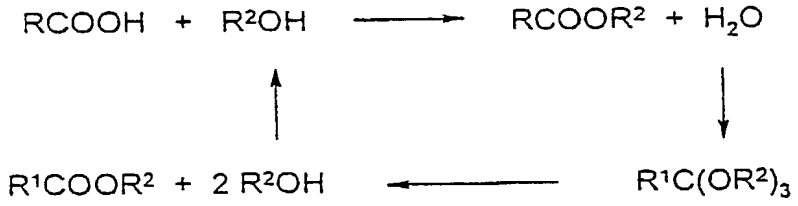
a) Removing water from the reaction equilibrium by addition of dehydrating salts (Kvittingen, L.; Sjursnes, B. and Anthonsen, T. *Tetrahedron* **1992**, *48*, 2793-2802). The drawback of the process is that the collisions between the salt particles and the enzyme ones damage the latter, thus reducing the life times and making their recovery difficult.

b) Removing water from the equilibrium by addition of molecular sieves (Fonteyn, F.; Blecker, C.; Lognay, G.; Marlier, M. and Severin, M. *Biotechnol. Lett.* **1994**, *16*, 693-696). In addition to the above drawbacks, the alcohol also can be removed, particularly in case of low molecular alcohols.

c) Removing water by distillation. This method can be used only when water is the lower boiling component of the mixture; therefore it cannot be used with low boiling alcohols or solvents.

d) Recycle of the reaction products to increase their optical purity (Morrone, R.; Nicolosi, G.; Patti, A. and Piattelli, M. *Tetrahedron: Asymmetry* **1995**, *6*, 1773-1778). This method clearly increases the work up costs.

It has now been found, and this is the object of the invention, that when the reaction is carried out in the presence of orthoesters, the latter react with water formed during the reaction, making therefore the process irreversible.



DISCLOSURE OF THE INVENTION

The present invention therefore provides a process for the resolution of enantiomeric mixtures of a chiral carboxylic acid of formula

R-COOH,

wherein R is a hydrocarbon residue optionally containing one or more heteroatoms and optionally mono- or polysubstituted, comprising an esterification reaction of said carboxylic acid in an organic solvent, in the presence of a stereoselective hydrolase, characterized in that an orthoester of formula

$R^1$-C($OR^2$)$_3$,

in which $R^1$ is selected from H and $C_1$-$C_4$alkyl and $R^2$ is $C_1$-$C_8$alkyl or -$CH_2$-$C_{6-10}$aryl,

is used as the esterification reactive.

R is preferably the residue of an antiinflammatory arylpropionic acid such as (±)-(*R,S*)-2-(2-fluoro-4-biphenyl)-propionic, (±)-(*R,S*)-2-(3-benzoylphenyl)-propionic, (±)-(*R,S*)-2-(4-isobutylphenyl)-propionic, (±)-(*R,S*)-2-[4-(1-oxo-2-isoindolinyl)phenyl]propionic, (±)-(*R,S*)-2-[4-(2-thenoyl)phenyl]-propionic, (±)-(*R,S*)-2-(6-methoxy-2-naphthyl)-propionic acids.

$R^1$ is preferably selected from H, methyl, ethyl, n-propyl, n-butyl.

The stereoselective hydrolase is preferably a lipase from Candida antarctica, Candida cylindracea, Pseudomonas cepacia, Mucor miehei, Mucor javanicus, Aspergillus niger, swine pancreas, or a protease from Aspergillus subtilis.

The esterification reaction is generally carried out at a temperature of 0-50°C, preferably at 45°C. Similarly, a supercritical gas, such as $CO_2$, can be used as the reaction solvent.

Conveniently the process according to the invention comprises the step of adding to the reaction mixture, consisting of the carboxylic acid, the hydrolase and the organic solvent, an amount of water or of a alcohol with 1-8 carbon atoms equivalent to 1-5% mols compared with the mols of said chiral carboxylic acid. The reaction is thereby activated, which then proceeds thanks to the formation of the alcohol following reaction of the orthoester with the water formed during the esterification reaction.

The resulting suspension is kept under stirring at the optimal temperature for the enzyme used. The progress of the reaction can be monitored by the usual analytical methods known to those skilled in the art. When the desired conversion value, on which the desired enantiomeric excess of the products depends, has been reached, the reaction is stopped by filtering off the enzyme. The reaction products are then recovered by separation with procedures known to those skilled in the art.

Alternatively to the use of orthoesters, carbonates may also be used in the process of the invention.

The irreversibility of the esterification, carried out with the process of the invention, allows to prepare chiral acids in enantiopure form (in particular the enantiomer not preferred by the enzyme) by extending the reaction times up to conversion values higher than 50%.

Figure 1a shows the change of the optical purity of the unreacted substrate in the esterification of rac-flurbiprofen, depending on the reaction time, when using methanol, ethanol, propanol and butanol as alcohol, acetonitrile as solvent and a lipase from Candida antarctica (with R stereopreference). In Figure 1b it is reported the progress of the reaction, under the same operative

conditions, using orthoformate (respectively methyl, ethyl, propyl, butyl) as alcohol source.

When comparing the progress of the reaction with alcohols (Figure 1a) and that with orthoformates (figure 1b) it is easily evident that in normal esterification of flurbiprofen the ee of the unchanged substrate reaches a maximum value of 80-85 and then begins to drop.

In patent contrast, when orthoformates are used the ee value continues to increase by extending the incubation period and consequently the conversion value. With all the orthoformates tested, the ee value of the unreacted acid reaches 95-98%.

In Figure 2 it is reported the trend for the esterification in hexane of 2-methylvaleric acid in the presence of Candida cylindracea lipase (Stereopreference S). The esterification with alcohol (Figure 2a) shows the usual course of the reversible reactions and the ee of the residual acid decreased when conversion is extended much beyond 50%. The esterification with the use of orthoformates proceeded as an irreversible reaction (Figure 2b) and with the best of the four tested, tributyl orthoformate, the ee values of the remaining substrate obtained is >98.

Obviously, the method proposed here can be used not only in the resolution of chiral acids, but also in the esterification of achiral acids, particularly when they are very expensive, to increase the yield by pushing the equilibrium toward completion.

The following examples disclose the invention in more detail.

Example 1

Preparation of enantiopure S-flurbiprofen

Novozym 435(R) (lipase from Candida antartica) (100 g) was added to a solution of racemic flurbiprofen (41 mmol, 10 g) in $CH_3CN$ (1 l) containing tripropyl orthoformate (123 mmol, 26.5 ml) and 0.1 ml of n-propanol. The mixture was incubated at 45°C under shaking (300 rpm) and conversion and ee of unreacted flurbiprofen were followed by hplc using a Chirex R-NGLY & DNB (250 x 4.0 mm) column. After 6 days conversion had reached 60% and the reaction was stopped filtering off the enzyme. Removal of the solvent in vacuo left a residue that was partitioned between hexane and aq. $NaHCO_3$ (3 g in 200 ml of water). The organic phase was washed with water, dried over $Na_2SO_4$ and the solvent removed to afford 6.8 g of (-)-R-flurbiprofen propyl ester (yield 58%, ee 64%). $^1H$ NMR ($CDCl_3$): $\delta$ 0.89 (t, 3H, J=7Hz), 1.54 (d, 3H, J=7Hz), 1.65 (m,2H), 3.78 (q, 1H, J=7Hz), 4.06 (t, 2H, J=6Hz), 7.1-7.6 (m, 8H). Anal. Calcd for $C_{18}H_{19}FO_2$; C, 75.70; H, 6.69. Found: C. 75.62; H, 6.89.

Acidification of the aqueous phase with $H_2SO_4$ gave a precipitate of (+)-S-flurbiprofen (3.9 g, yield 39%, ee>98%). Anal. Calcd for $C_{15}H_{13}FO_2$; C, 73.76; H, 5.36. Found: C. 73.90; H, 5.52.

Example 2

Preparation of enantiopure (R)-2-Methylvaleric acid

Candida cylindracea lipase (50 g) was added to a solution of racemic 2-methylvaleric acid (86.2 mmol, 10 g) in hexane (500 ml) containing tributyl orthoformate (86.2 mmol, 23 ml) and 0.1 ml of n-butanol. The mixture was incubated at 45°C under shaking (300 rpm). Conversion and ee of the butyl ester were followed by GC using a ß-cyclodextrin (dimethylpenthylbetacdx/OV1701 3:7) column. After 48 h conversion had reached 65% and reaction was

stopped filtering off the enzyme. After partition with aq. $NaHCO_3$ (3 g in 200 ml of water) the hexane phase was dried over $Na_2SO_4$ and evaporated under vacuum to furnish 9.6 g of (S)-2-methylvaleric butyl ester (yield 65%, ee 53%). MS data agreed with those reported in the literature (Kim Ha, J.; Lindsay, R.C.; J. Food Compos. Anal. 1989, 2, 118-131). Anal. Calcd for $C_{10}H_{20}O_2$; C, 69.72; H, 11.70. Found: C. 69.98; H, 11.84.

The aqueous phase was acidified with $H_2SO_4$, extracted three times with hexane and the organic phase were pooled. Removing of hexane under vacuum gave 3.5 g of (R)-2-methylvaleric acid (yield 35%, ee>97%). $[a]_D^{20}$= 18.2 (neat); (lit. $[a]_D^{20}$= 18.4 (neat); Levene, P. A.; Marker, R. E. J. Biol. Chem. 1932, 98,1) Anal. Calcd for $C_6H_{12}O_2$; C, 62.04; H, 10.41. Found: C. 62.31; H, 10.52.

CLAIMS

1. A process for the resolution of enantiomeric mixtures of a chiral carboxylic acid of formula

R-COOH,

wherein R is a hydrocarbon residue optionally containing one or more heteroatoms and optionally mono- or polysubstituted, comprising an esterification reaction of said carboxylic acid in an organic solvent, in the presence of a stereoselective hydrolase, characterized in that an orthoester of formula

$R^1$-C($OR^2$)$_3$,

in which $R^1$ is selected from H and $C_1$-$C_4$alkyl and $R^2$ is $C_1$-$C_8$alkyl or -$CH_2$-$C_{6-10}$aryl,

is used as the esterification reactive.

2. A process as claimed in claim 1, wherein $R^1$ is selected from H, methyl, ethyl, *n*-propyl, *n*-butyl.

3. A process as claimed in claim 2, wherein said stereoselective hydrolase is a lipase selected from Candida antarctica, Candida cylindracea, Pseudomonas cepacia, Mucor miehei, Mucor javanicus, Aspergillus niger, swine pancreas, or a protease from Aspergillus subtilis.

4. A process as claimed in any one of the above claims, wherein said esterification reaction is carried out at a temperature of 0-50°C, preferably at 45°C.

5. A process as claimed in any one of the above claims comprising the step of adding the reaction mixture with an amount of water or of a alcohol with 1-8 carbon atoms equivalent to 1-5% mols compared with the mols of said chiral carboxylic acid.

6. A process as claimed in any one of the above claims, wherein in said esterification reaction the meso form of a bicarboxylic acid is used as substrate.

7. A process as claimed in the above claims 1-6, wherein said carboxylic acid is selected from (±)-(*R,S*)-2-(2-fluoro-4-biphenyl)-propionic, (±)-(*R,S*)-2-(3-benzoylphenyl)-propionic, (±)-(*R,S*)-2-(4-isobutylphenyl)-propionic, (±)-(*R,S*)-2-[4-(1-oxo-2-isoindolinyl)phenyl]propionic, (±)-(*R,S*)-2-[4-(2-thenoyl)phenyl]-propionic, (±)-(*R,S*)-2-(6-methoxy-2-naphthyl)-propionic acids.

8. The use of an orthoester of formula

$$R^1\text{-}C(OR^2)_3,$$

in which $R^1$ is selected from H and $C_1$-$C_4$alkyl and $R^2$ is $C_1$-$C_8$alkyl or -$CH_2$-$C_{6-10}$aryl,

in combination with a stereoselective hydrolase in the resolution of enantiomeric mixtures of carboxylic chiral acids.

9. The use as claimed in claim 8, wherein said hydrolase is a lipase selected from Candida antarctica, Candida cylindracea, Pseudomonas cepacia, Mucor miehei, Mucor javanicus, Aspergillus niger, swine pancreas, or a protease from Aspergillus subtilis.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 February 2001 (01.02.2001)

PCT

(10) International Publication Number
WO 01/07564 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/EP00/07102

(22) International Filing Date: 25 July 2000 (25.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
ME99A000005 26 July 1999 (26.07.1999) IT

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

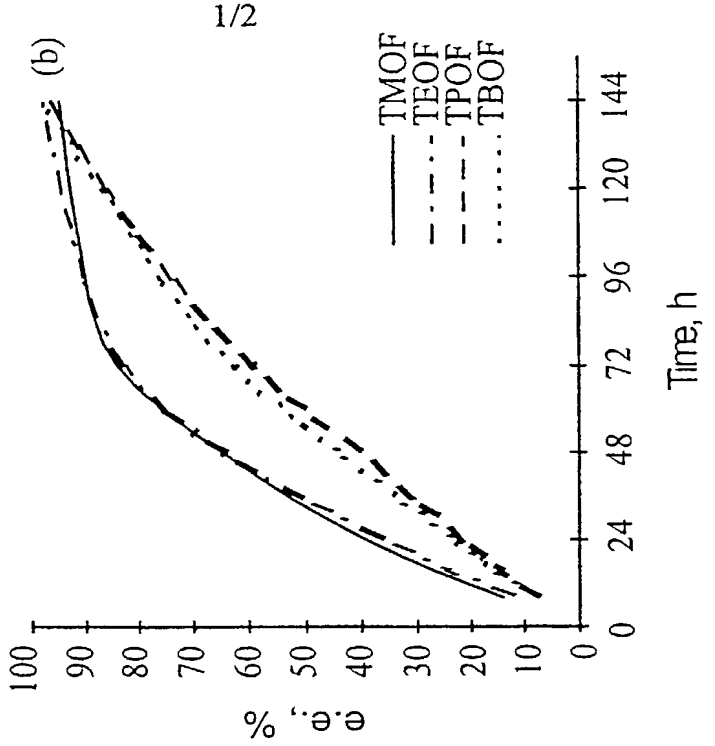
**Published:**
— *Without international search report and to be republished upon receipt of that report.*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/07564 A2

(54) **Title:** THE USE OF ORTHOESTERS FOR THE SYNTHESIS OF CHIRAL ACIDS IN BIOCATALYZED IRREVERSIBLE ESTERIFICATION PROCESSES

(57) **Abstract:** A process for the resolution of enantiomeric mixtures of a chiral carboxylic acid, comprising an esterification reaction of said carboxylic acid in an organic solvent, in the presence of a stereoselective hydrolase, characterized in that an orthoester of the formula: $R^1$-$C(OR^2)_3$, in which $R^1$ is selected from H and $C_1$-$C_4$alkyl and $R^2$ is $C_1$-$C_8$alkyl or -$CH_2$-$C_{6-10}$aryl, is used as the esterification reactive.



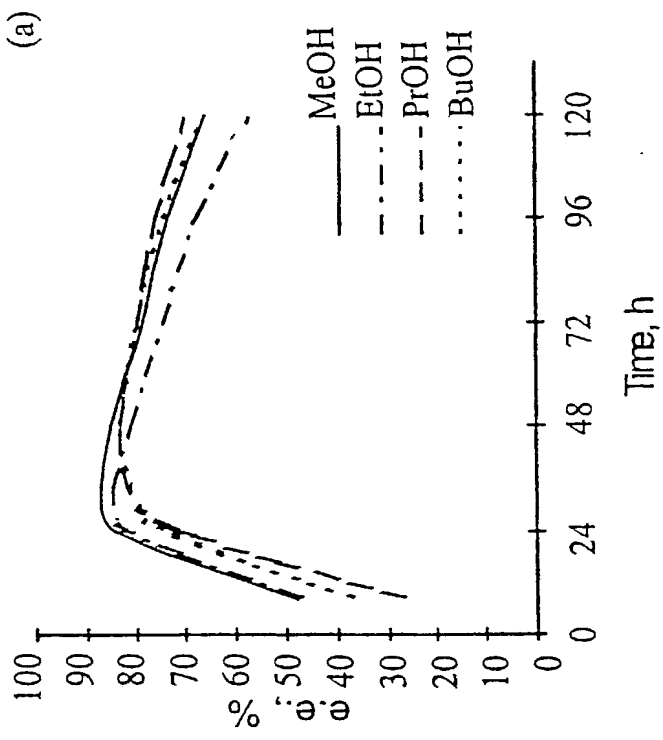




**Fig. 1.** Enantiomeric excess (ee) value of unreacted Flurbiprofen versus reaction time with different alcohols (a) and orthoformates (b)

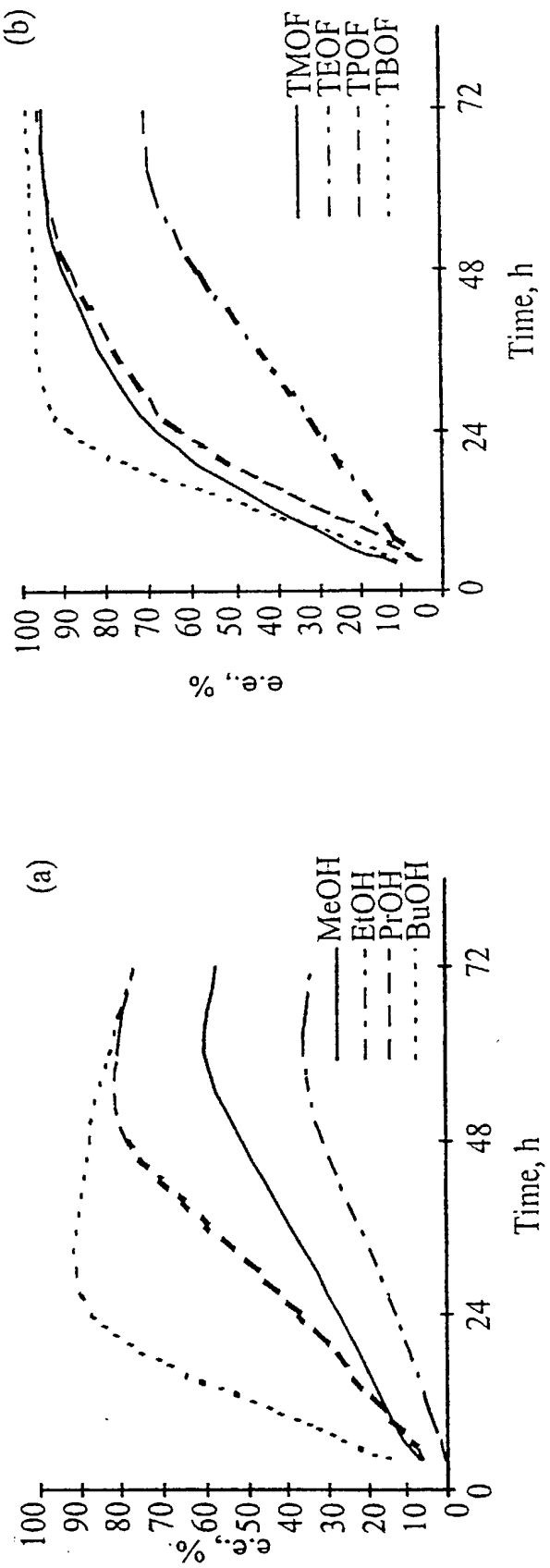



**Fig. 2.** Enantiomeric excess (ee) value of unreacted 2-Methylvaleric acid versus reaction time with different alcohols (a) and orthoformates (b)

Ref. _____

# COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

The use of orthoesters for the synthesis of chiral acids in biocatalyzed irreversible esterification processes

the specification of which: *(check one)*

**REGULAR OR DESIGN APPLICATION**

[ ] is attached hereto.

[ ] was filed on _____ as application Serial No. _____ and was amended on _____ (if applicable).

**PCT FILED APPLICATION ENTERING NATIONAL STAGE**

[X] was described and claimed in International application No. PCT/EP00/07102 filed on 25.07.2000 and as amended on _____ (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

**PRIORITY CLAIM**

I hereby claim foreign priority benefits under 35 USC 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

**PRIOR FOREIGN APPLICATION(S)**

| Country | Application Number | Date of Filing (day, month, year) | Priority Claimed |
|---|---|---|---|
| Italy | ME99A000005 | 26.07.1999 | YES |
| | | | |

*(Complete this part only if this is a continuing application.)*

I hereby claim the benefit under 35 USC 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 USC 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing Date) (Status--patented, pending, abandoned)

## POWER OF ATTORNEY

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from ____ _____ as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

As a named inventor, I hereby appoint the following attorney(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: **Robert J. PATCH, Reg. No. 17,355, Andrew J. PATCH, Reg. No. 32,925, Robert F. HARGEST, Reg. No. 25,590, Benoît CASTEL, Reg. No. 35,041, Eric JENSEN, Reg. No. 37,855, and Thomas W. PERKINS, Reg. No. 33,027**, c/o YOUNG & THOMPSON, Second Floor, 745 South 23rd Street, Arlington, Virginia 22202.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1 of 3

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